Photodiagnosis and Photodynamic Therapy (2013) 10, 252–259

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Monoglycoconjugated phthalocyanines: Effect of sugar and linkage on photodynamic activity

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Available online 19 February 2013

KEYWORDS
Photodynamic therapy;
Photosensitiser;
Phthalocyanine;
Carbohydrate;
Click chemistry

Summary
Background: Click chemistry can be advantageously used to graft carbohydrates on phthalocyanines which are potent photosensitisers, but the effect of the presence of triazole moieties on photodynamic efficiency was not investigated systematically to date. The nature and linkage of the sugar were investigated in order to define structure–activity relationships.
Method: Two sets of monoglycoconjugated water-soluble phthalocyanines have been designed and their photodynamic activity and uptake investigated in HT-29 human colon adenocarcinoma cells. Carbohydrates: galactose, mannose or lactose were grafted onto Zn(II) phthalocyanines either by glycosylation or by click reaction.
Results: The triazole linkage formed by click conjugation lowered the biological efficiency for mannose and galactose, compared to classical glycosylation grafting. The mannose conjugate formed by glycosylation was the most photodynamically active, without correlation with the photosensitiser cell uptake.

Introduction

Photodynamic therapy (PDT) is a non-invasive treatment for a number of diseases, including cancer [1], age-related macular degeneration [2] and chronically infected wounds and ulcers [3]. The clinical process of PDT involves administering a photosensitiser which localises in the diseased tissue. Excitation of the photosensitiser *in situ*, using visible

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http://dx.doi.org/10.1016/j.pdpdt.2012.11.009

or near infrared light results in energy transfer, *via* the excited triplet state, to surrounding molecular oxygen, which is converted to its excited singlet state, the toxic species that causes oxidative damage to proximal biological components. If sufficient damage occurs this leads to cell death by either necrosis or apoptosis. During the last four decades this method has been clinically developed, and major research efforts have been devoted to the design and synthesis of better photosensitisers [4]. The current third-generation of photosensitisers exhibit most of the required physicochemical properties [5], and are now being combined with imaging [6] or targeting [7] moieties for theranostic and selectivity enhanced PDT. Phthalocyanines, due to their maximum absorption band in the far red and advantageous photophysical properties, are appealing PDT sensitisers [8].

Grafting carbohydrates on to photosensitisers offers several advantages: besides the hydrophilicity of such moieties which aids delivery in aqueous systems, potential selective recognition and/or enhanced cell uptake by cancer cells having elevated receptor densities for these species are possible. Carbohydrates have been grafted on to nanoparticle-photosensitiser constructs, as in the case of mannose-targeted mesoporous silica nanoparticles [9], or directly onto photosensitisers, including chlorins [10], porphyrins [11] and phthalocyanines [12]. Associated synthetic methods, such as the development of new glycosylation procedures [13] or the use of click chemistry, have also been reported [14].

The present work studies the effects of two parameters that are of importance in the chemistry of carbohydrate-substituted phthalocyanines, and have not been investigated systematically so far: the nature of the carbohydrate and of the grafting mode.

Material and methods

Dichloromethane used for glycosylation reactions was washed twice with water, dried with $CaCl_2$ and distilled from P_2O_5. CH_2Cl_2 was stored over 4 A. Thin layer chromatography was performed on aluminium sheets coated with Silica gel 60 F254 (Alugram). Compounds were visualised by spraying the TLC plates with dilute 15% aq H_2SO_4, followed by charring at 150 °C for a few min. Column chromatography was performed on Silica-gel (Silicycle). NMR spectra were recorded with Bruker ALS300 or DRX400 spectrometers or on a Varian 500 MHz spectrometer. For ATR-IR spectra acquisition, the ATR accessory (PIKE Technologies) was accommodated in the sample compartment of a Vertex 80 FTIR spectrometer (internal reflection element: ZnSe crystal mounted onto a stainless steel plate, resolution: 4 cm^{-1}. The phthalocyanine solution in chloroform was applied directly onto the upper face of a horizontal sampling ATR accessory. The spectrum was recorded after chloroform evaporation). Optical absorption spectra in the UV—visible region were recorded with a Schimadzu 2001 UV spectrophotometer. The HPLC system is an Agilent 1100 series HPLC system (ChemStation software) equipped with a G 1311A pump and G1315B diode array detector. For protected compounds, a normal phase column Lichrosorb-SI-60 (4.6 mm × 250 mm) (Alltech Associates, Inc.) was used. The samples were dissolved in $CHCl_3$ at a concentration of 0.2 mg mL^{-1} for the normal phase. Solvent system $CHCl_3$-THF (70/30), flow rate 0.9 mL min^{-1}, at 700 nm detection. For final unprotected compounds, a reverse phase column Shim-pack ODS (4.0 mm × 250 mm) (Schimadzu, Japan) was used. The samples were dissolved in water at a concentration of 0.2 mg mL^{-1} for the reverse phase. Solvent system acetonitrile in H_2O, 0—100% in 30 min, flow 0.8 mL min^{-1}, at 700 nm detection. The high resolution mass spectra were recorded in positive ion mode on a hybrid quadrupole time-of-flight mass spectrometer (MicroTOFQ-II, Bruker Daltonics, Bremen) with an Electrospray Ionisation (ESI) ion source. The gas flow of spray gas was 0.6 bar and the capillary voltage was 4.5 kV. The solutions were infused at 180 μL h^{-1} in a mixture of solvents (methanol/dichloromethane/water 45/40/15). The mass range of the analysis was 50—3000 *m/z* and the calibration was done with ESI tune mix Agilent.

General method for the glycosylation reaction. The phthalocyanine **5** (90 mg, 77.5 μmol) and the perbenzoylated-D-glycopyranosyl trichloroacetimidate (115 mg, 155.0 μmol for monosaccharides **7** and **8**, 188 mg, 155.0 μmol for lactosyl imidate **9**, 2 equiv.) were dissolved in dry CH_2Cl_2 (5.0 mL) in the presence of crushed activated 4 Å molecular sieves (250 mg) and the suspension was cooled to −10 °C with stirring under an argon atmosphere. Trimethylsilyl trifluoromethanesulfonate (30 μL, 2 equiv. relatively to the phthalocyanine) was added and the mixture was stirred and the temperature was allowed to rise to 0 °C. The mixture was stirred overnight, before neutralisation with triethylamine (30 μL) and filtration on celite. The organic phase was concentrated; the product was dissolved 80% in aqueous acetic acid and the solution was stirred for 5 h at 70 °C. After concentration *in vacuo*, and coevaporation twice from toluene (2 mL × 5 mL), the residue was de-*O*-benzoylated overnight with a catalytic amount of sodium methoxide in methanol (20 mL); the reaction mixture was neutralised with acetic acid and concentrated again. The crude product was acetylated overnight in a 2:1 pyridine—acetic anhydride mixture (5 mL). After concentration and coevaporation from toluene, the residue was purified by column chromatography, by elution first with EtOAc-petroleum ether (9:1 for monosaccharides, 3:1 for the disaccharide, for elimination of the excess of sugars), then with CH_2Cl_2—EtOH (20:1).

(1(4),8(11),15(18)-Tri-(2,3-di-*O*-acetyl-1-glyceryloxy)-23(24)-(12-(1,4,7,10-tetraoxa dodecyl) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside)phthalocyaninato zinc(II) (10). Obtained as described above from 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (**7**) (115 mg) and the phthalocyanine **5** (90 mg). Compound **10** was recovered in 83% yield (103 mg) after purification by column chromatography. Deep blue powder; R_f 40 (20:1 CH_2Cl_2—EtOH); ^{13}C NMR (DMSO-d_6): δ = 171.23—169.55 (CH_3COO), 167.72—105.75 (aromatic C), 101.16 (C-1) ppm. ATR-IR: υ = 3060, 2990, 1736, 1605, 1488, 1453, 1369, 1276, 1261, 1218, 1044, 960, 898, 764, 750 cm^{-1}; HRMS-ESI: *m/z* $(M+H)^+$ calcd for $C_{75}H_{81}N_8O_{29}Zn$, 1621.4395; found 1621.4350; HPLC *R*t: 13 min; UV/Vis (DMSO): λ_{max} (log ε) = 697 (5.04), 627 (4.34), 351(4.47).

(1(4),8(11),15(18)-Tri-(2,3-di-*O*-acetyl-1-glyceryloxy)-23(24)-(12-(1,4,7,10-tetraoxa dodecyl) 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside))phthalocyaninato zinc(II) (11). Obtained as described above from 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**8**) (115 mg) and the phthalocyanine **5** (90 mg). Compound **11** was recovered in 85% yield (106 mg) after purification by column chromatography. Deep blue powder; R_f 40 (20:1 CH_2Cl_2—EtOH); ^{13}C NMR (DMSO-d_6): δ = 171.23—169.55 (*C*H_3COO), 163.30—105.35 (aromatic C), 98.16 (C-1) ppm. ATR-IR: υ = 3060, 2990, 1736, 1605, 1488, 1453, 1369, 1276, 1261, 1218, 1044, 960, 898, 764, 750 cm^{-1}; HRMS-ESI: *m*/*z* (M+H)$^+$ calcd for $C_{75}H_{81}N_8O_{29}Zn$, 1621.4395; found 1621.4414; HPLC *R*t: 12 min; UV/Vis (DMSO): λ_{max} (log ε) = 697 (5.18), 627 (4.45), 351(4.62).

(1(4),8(11),15(18)-Tri-(2,3-di-*O*-acetyl-1-glyceryloxy) -23(24)-(12-(1,4,7,10-tetraoxa dodecyl) 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside)) phthalocyaninato zinc(II) (12). Obtained as described above from 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate (**9**) (188 mg) and the phthalocyanine **5** (90 mg). Compound **12** was recovered in 65% yield (96 mg) after purification by column chromatography. Deep blue powder; R_f 40 (20:1 CH_2Cl_2—EtOH); ^{13}C NMR (DMSO-d_6): δ = 171.31—169.06 (*C*H_3COO), 161.51—105.80 (aromatic C), 101.26, 100.86 (C-1, C-1′) ppm. ATR-IR: υ = 2989, 2940, 2882, 1739, 1606, 1588, 1489, 1369, 1275, 1260, 1216, 1043, 955, 900, 764, 749 cm^{-1}; HRMS-ESI: *m*/*z* (M+H)$^+$ calcd for $C_{87}H_{97}N_8O_{37}Zn$, 1909.5241; found 1909.5161; HPLC *R*t: 16 min; UV/Vis (DMSO): λ_{max} (log ε) = 697 (5.14), 627 (4.41), 351(4.50).

General method for phthalocyanine deprotection. Phthalocyanines **10—12** were treated for 2 days at rt in a 2:1:1 MeOH—H_2O—Et_3N mixture (20 mL). After concentration *in vacuo* and coevaporation twice from H_2O (10 mL), the residue was purified by column chromatography.

(1(4),8(11),15(18)-Tri-(1-glyceryloxy)-23(24)-(12-(1,4,7,10-tetraoxadodecyl) β-D-galactopyranoside)) phthalocyaninato zinc(II) (1-Gal). Obtained as described above from **10** (100 mg). Compound **1-Gal** was recovered in 85% yield (62 mg) after purification by column chromatography using 65:25:4 $CHCl_3$—MeOH—H_2O as the eluent. Deep blue powder; R_f 0.30 (65:25:4 $CHCl_3$—MeOH—H_2O); ATR-IR: υ = 3269, 2944, 2881, 1605, 1586, 1490, 1451, 1393, 1334, 1273, 1235, 1034, 948, 796, 747 cm^{-1}; HRMS-ESI: *m*/*z* (M+H)$^+$ calcd for $C_{55}H_{61}N_8O_{19}Zn$, 1201.3339; found 1201.3305; HPLC *R*t: 21 min; UV/Vis (DMSO): λ_{max} (log ε) = 702 (5.046), 633 (3.93), 343 nm (4.10).

(1(4),8(11),15(18)-Tri-(1-glyceryloxy)-23(24)-(12-(1,4,7,10-tetraoxadodecyl) α-D-mannopyranoside)) phthalocyaninato zinc(II) (1-Man). Obtained as described above from **11** (103 mg). Compound **1-Man** was recovered in 81% yield (61 mg) after purification by column chromatography using 65:25:4 $CHCl_3$—MeOH—H_2O as the eluent. Deep blue powder; R_f 0.30 (65:25:4 $CHCl_3$—MeOH—H_2O); ATR-IR: υ = 3272, 2930, 2884, 1643, 1605, 1587, 1488, 1453, 1385, 1336, 1269, 1238, 1100, 1058, 948, 880, 800, 742 cm^{-1}; HRMS-ESI: *m*/*z* (M+H)$^+$ calcd for $C_{55}H_{61}N_8O_{19}Zn$, 1201.3339; found 1201.3329; HPLC *R*t: 21 min; UV/Vis (DMSO): λ_{max} (log ε) = 702 (4.57), 633 (3.98), 343 nm (3.88).

(1(4),8(11),15(18)-Tri-(1-glyceryloxy)-23(24)-(12-(1,4,7,10-tetraoxadodecyl) 4-*O*-β-D-galactopyranosyl)-β-D-glucopyranoside)) phthalocyaninato zinc(II) (1-Lac). Compound 1-Lac was prepared as described above from **10** (90 mg), but due to its high polarity, the expected product did not migrate on column chromatography: elution with 65:25:4 $CHCl_3$—MeOH—H_2O as the eluent allowed the separation of the unreacted phthalocyanine **5**, and product **1-Lac** was adsorbed on silica-gel and recovered on the top of the column. After extraction with 4:1 dichloromethane—methanol (4 mL × 50 mL) and concentration, the product was filtrated on a short column of celite with 65:25:4 $CHCl_3$—MeOH—H_2O as eluent. Product was obtained in 78% yield (51 mg). Deep blue powder; R_f 0.10 (65:25:4 $CHCl_3$—MeOH—H_2O); ATR-IR: υ = 3286, 2937, 2874, 1654, 1589, 1486, 1451, 1380, 1338, 1271, 1240, 1035, 742 cm^{-1}; HRMS-ESI: *m*/*z* (M+H)$^+$ calcd for $C_{61}H_{71}N_8O_{24}Zn$, 1363.3867; found 1363.3884; HPLC *R*t: 20 min; UV/Vis (DMSO): λ_{max} (log ε) = 702 (4.17), 633 (3.56), 343 nm (3.88).

In vitro photodynamic activity

Phthalocyanines were formulated in DMSO and diluted in medium (McCoy's 5A + 2 mL L-glutamine) to give concentrations ranging from 5×10^{-4} to 1×10^{-6} M, when added to HT-29 cells (Human Caucasian colon adenocarcinoma, 1×10^{-6} cells mL^{-1}). The cells were then incubated in the dark for 1 h at 37 °C in a 5% CO_2 atmosphere then washed with a 3 fold excess of medium to eliminate any unbound phthalocyanine. Pellets of cells were re-suspended in 1 mL of medium and aliquots (4 × 100 μL) transferred to 96 well plates in duplicate. One plate was irradiated with red light (>600 nm; 3.6 J cm^{-2}) at a fluence rate of 4.6 mW cm^{-2}, while the other plate served as a drug/no-light control. After irradiation, 5 mL of Foetal Bovine Serum was added to each well and the plates were returned to the incubator. After 24 h, an MTT cell viability assay was performed and the results expressed as % of cell viability *vs.* phthalocyanine conjugate concentration. Each experiment was performed in triplicate.

Cell uptake

200 μL of each phthalocyanine was formulated in DMSO and diluted in medium (McCoy's 5A + 2 mM L-glutamine) to the concentration previously determined to result in 50% cell death upon irradiation (LD_{50}). This was added to 800 μL of HT-29 cells (Human Caucasian colon adenocarcinoma) adjusted to a concentration of 1×10^6 cells/mL. The cells were incubated in the dark for an hour at 37 °C in a 5% CO_2 atmosphere, after which they were washed in a 3× excess of medium to eliminate any unbound phthalocyanine. The resulting cell pellets were re-suspended in 1 mL medium and 3× 300 μL aliquots were transferred into a black walled 96 wells plate. The plate was put into a freezer (−18 °C) overnight to induce cell disruption. The next day, the plate was thawed in an incubator (37 °C) for 35 min after which the contents of each well were thoroughly dispersed. The content of each well was then diluted with DMSO (1:3) and transferred into a new black-walled 96 wells plate.

Fluorescence was read using a Varian Cary Eclipse Fluorescence Spectrophotometer fitted with plate reading module, and the phthalocyanine concentrations read from previously obtained fluorescence standard curves (Exc: 690 nm, Em: 700—760 nm, Scan speed: Slow, Slits: 5 nm, PMT: Manual 710 V).

Results and discussion

Molecular design

Recently, we established the benefits of using glycerol in non-peripheral positions on the macrocycle of Zn phthalocyanines [15]. We designed and reported several modified derivatives, all having the common non-peripheral glycerol substitution pattern [16]. The two sets of molecules prepared for this study are presented in Fig. 1. Their design aims to investigate two structural parameters. The nature of the carbohydrate being the first parameter: β-D-galactose, α-D-mannose and β-D-lactose have been selected as they are among the main energy providers for cells. Secondly, due to the rising role of click chemistry as a synthetic tool, especially in carbohydrate chemistry [17], a comparison of the effect of grafting the sugar either *via* a glycosidic bond or a triazole bridge was performed, in order to determine the effect of the triazole linkage on PDT efficiency. In the first set **1-Sug**, composed of **1-Gal**, **1-Man** and **1-Lac**, the carbohydrates are all grafted to the phthalocyanine *via* a glycosidic bond, while in the second set **2-Sug**, composed of **2-Gal**, **2-Man**, **2-Lac**, the carbohydrates are grafted to the phthalocyanine *via* a triazole bridge. To avoid possible steric hindrance of the phthalocyanine macrocycle during carbohydrate recognition, the carbohydrate moieties were grafted at the extremity of a tetraethylene glycol spacer, a substituent known to enhance water-solubility [8b], [18].

Synthesis and characterisation

The glycosylated phthalocyanines **1-Sug** and **2-Sug** were synthesised from the phthalocyanines **5** or **6** either by a glycosylation reaction or by a click reaction. The required phthalocyanines **5** and **6** were prepared as previously described [14d] by a mixed condensation of solketal [15] and tetraethyleneglycol [18] substituted phthalonitriles (respectively, **3** and **4**) in the presence of $Zn(OAc)_2$, leading to phthalocyanine **5** which could be converted easily in two steps into **6** [14d]. Phthalocyanines exhibiting this so-called AB_3 unsymmetrical substitution pattern can be advantageously prepared in high yields under these mixed condensation conditions [19].

Glycosylation reactions leading to protected derivatives of compounds **1-Sug** were performed in dichloromethane at −10 °C on the acceptor **3** using 2,3,4,6-tetra-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate (**7**) [20], 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate (**8**) [21] and 4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**9**) [22], respectively, resulting in the corresponding glycosylated phthalocyanines (Scheme 1). The general procedure for the imidate glycosylation reaction suggests it is enhanced by a catalytic amount of promoter [23]. In the present case, an excess of (trimethylsilyl trifluoromethanesulfonate) was necessary to complete the reaction. This could be explained by the basic character of the phthalocyanine **5** which partially neutralises the

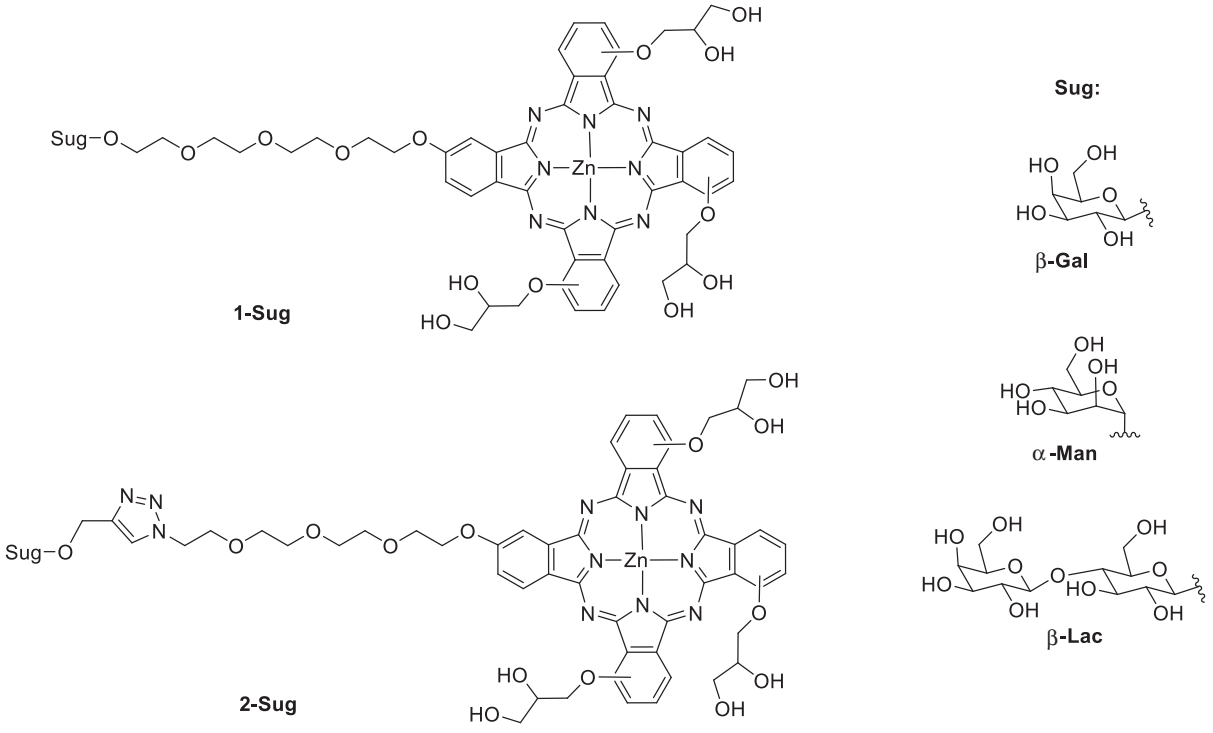
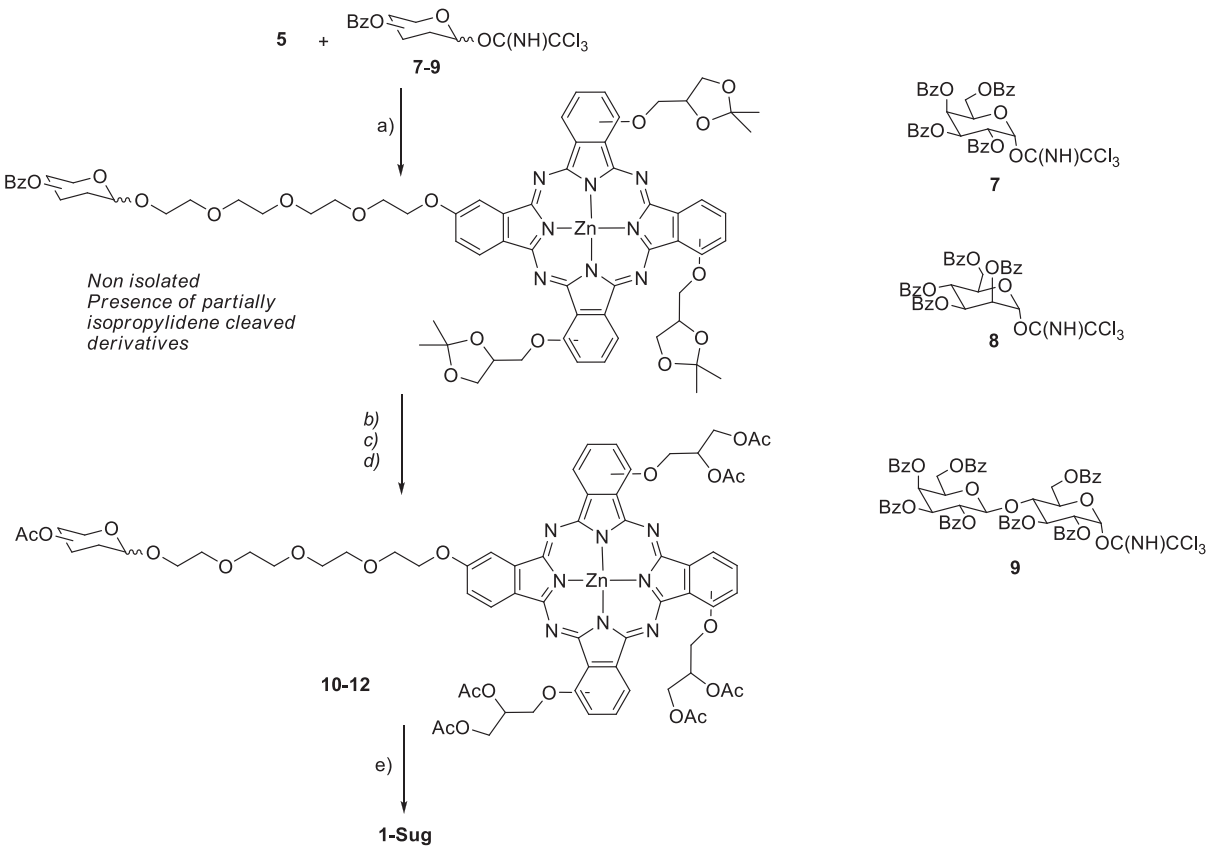


Figure 1 Structure of the phthalocyanines **1-Sug** and **2-Sug**.



Scheme 1 (a) TMSOTf (2 equiv.), −10 °C, CH_2Cl_2, 16 h; (b) 80% AcOH, 70 °C, 6 h; (c) MeONa, MeOH; (d) Ac_2O, C_5H_5N; (e) 2:1:1 MeOH—H_2O—Et_3N, 2 d, rt.

promoter. At the same time, we observed a change in the colour of the reaction mixture, from deep green to brownish under acidic conditions. Since partial isopropylidene cleavage occurs during the glycosylation reaction, the crude products were directly treated with 80% acetic acid in order to cleave the remaining isopropylidene groups; then, the mixture was fully de-*O*-benzoylated and reacetylated. The fully acetylated phthalocyanine derivatives were recovered after purification by column chromatography in 85% yield for monosaccharidic **10** and **11**, and 65% for disaccharidic **12**. After complete de-*O*-acetylation in a 2:1:1 MeOH—H_2O—Et_3N mixture, the products were purified by column chromatography and at this stage, we observed the presence of about 5% unreacted fully deprotected phthalocyanine **5**, which could not be detected during the previous step, and which was now separable from the product. Pure products **1-Gal**, **1-Man** and **1-Lac** were isolated in 78—85% yields (Scheme 1).

Compounds **10**—**12** all exhibited molecular peaks on high resolution ESI mass spectroscopy. Their ^{1}H NMR spectra were too complex to be interpreted, as it is often the case in tetrasubstituted phthalocyanines which are regioisomeric mixtures. In addition, in the case of the molecules prepared in these works, expected peaks of the substituents are overlapping. Nevertheless, ^{13}C NMR showed the expected peak corresponding to the anomeric carbons. Complete deprotection of **10**—**12** was indicated by disappearance of the carbonyl peaks on the ATR-IR spectra of **1-Sug** around 1700 cm^{-1}. **1-Sug** have also been analysed by high resolution ESI mass spectroscopy, each compound exhibiting molecular peaks fitting the theoretical isotopic pattern (Fig. 2). Due to insufficient solubility of the deprotected compounds **1-Sug** at concentrations required for NMR experiments, suitable spectra could not be registered. Purity of compounds was established by analytic HPLC experiments.

The synthesis of the **2-Sug** series was achieved by ''clicking'' the appropriate alkynyl substituted carbohydrates onto **6** [14d] as previously described on similar derivatives [14b]. If carbohydrates used for the glycosylation of set **1-Sug** were benzoylated as this protecting group is suitable for the preparation of trichloroacetimidate donors, alkynyl substituted carbohydrates used for the click reaction of set **2-Sug** are acetylated. Click reactions were performed under biphasic conditions in a dichloromethane—water mixture from the azidophthalocyanine **6** and were promoted by sodium ascorbate and copper sulfate. The resulting phthalocyanines were deprotected in two steps: firstly acidic hydrolysis of the acetals (80% acetic acid at 70 °C over 6 h), then removal of the acetyl groups in a 2:1:1 methanol—water—triethylamine mixture.

In vitro photodynamic activity

The potential of the series of glycosylated phthalocyanines described above as photodynamic sensitisers was evaluated

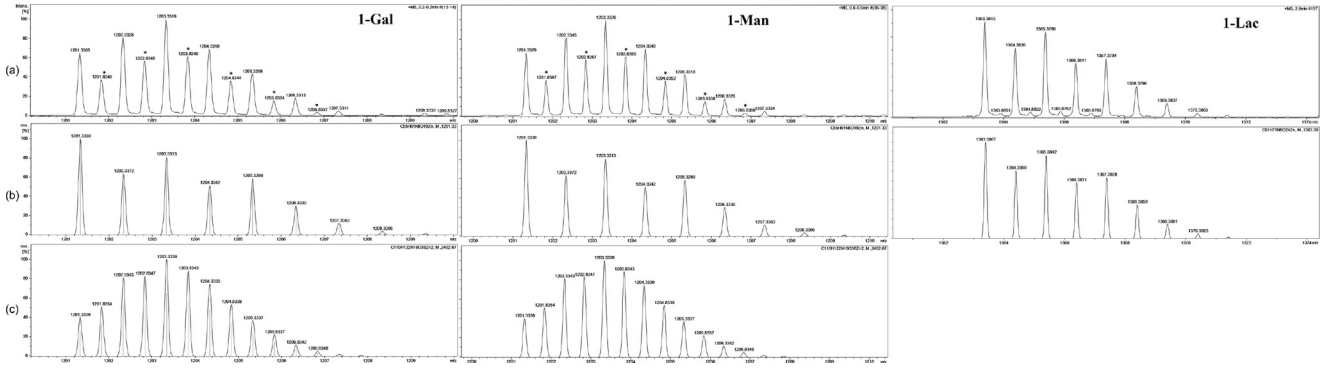


Figure 2 Experimental (a), theoretical monocharged (b) and theoretical dicharged non covalent dimer (c) HRMS-ESI spectra of **1-Gal**, **1-Man** and **1-Lac**. Stars indicated peaks corresponding to the non-covalent dimer dicharged. The dicharged non covalent dimer of **1-Lac** was not observed in these conditions.

on the human colon adenocarcinoma cell line (HT-29). Cells were incubated with varying concentrations of each phthalocyanine for 1 h and then excess photosensitiser was removed by repeated washing. Cells were then irradiated with red light (>600 nm; 3.7 J cm^{-2}) and incubated for 24 h before cell viability was assessed by MTT assay. Concentrations required to kill 50% of cells (LD_{50}) under these standard conditions were then calculated from cell survival curves (Table 1 and Fig. 3). All glycosylated phthalocyanines tested gave LD_{50} values in the micromolar range with the exception of **1-Lac**, which failed to give an LD_{50} at the highest concentration used (500 μM). The most potent compound was the directly grafted mannose conjugate **1-Man** with an LD_{50} value of 110 μM. Comparison of the effect of direct *vs* click grafting of sugars to the common phthalocyanine on photodynamic activity, indicated significant loss of activity for both galactose and mannose conjugates in going from direct to click linkage, a factor that was most marked for the mannose conjugate, which suffered a loss in activity of greater than three fold. These results of a negative effect of triazole linkage are in accordance with previously reported observations on porphyrin derivatives [14f]. The lowered activity for galactose and mannose conjugates formed by click attachment may point towards involvement of receptor binding, and greater disruption of this interaction due to the proximity of the bulky triazole to the sugar. Receptor-mediated

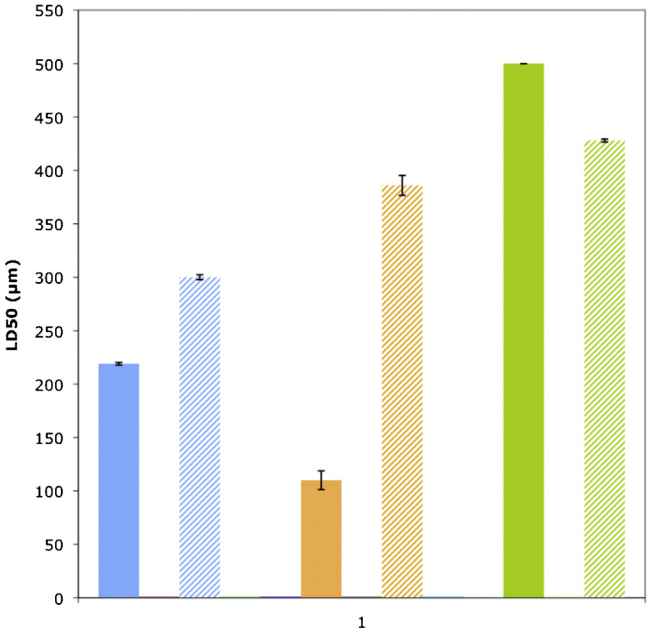


Figure 3 Comparative representation of LD_{50} concentration (μM) for 1-Sug (full colour) and 2-Sug (dashed colour) derivatives. Gal in blue, Man in orange, Lac in green.

Table 1 LD_{50} and related intracellular concentrations.

Phthalocyanine	LD_{50} concentration (μM) (standard deviation in brackets)	Intracellular concentration (pM) per cell at LD_{50} value (standard deviation in brackets)
1-Gal	219 (1.2)	14.6 (1)
2-Gal	300 (2.1)	14.7 (1.4)
1-Man	110 (8.8)	11.7 (1.1)
2-Man	386 (9.4)	17.2 (2.1)
1-Lac	>500	26.7 (1.9)
2-Lac	428 (1.4)	66.3 (2.4)

uptake of mannose—nanoparticle conjugates has recently been reported lending support to this postulate [9a].

Correlation of photodynamic activity with cellular uptake failed to show a direct link between potency of cell killing and photosensitiser uptake. Similar levels of uptake (11.7—17.2 pM per cell; Table 1) were observed for galactose and mannose conjugates formed by both direct glycosylation and click conjugation, however, these intracellular levels of photosensitiser conjugates resulted in significantly different photodynamic activities. Interestingly, the conjugate with the greatest photodynamic activity, **1-Man**, corresponded with the lowest intracellular uptake of all the conjugates tested, suggesting that bulk sensitiser uptake is not the major factor involved in photodynamic damage. Differences in uptake for galactose and mannose conjugates formed by direct grafting *vs* click linkage were relatively small, 0.1 and 5.5 pM per cell, respectively, indicating that absolute uptake

is relatively insensitive to the nature of the conjugation linkage. The lactose conjugates exhibited both the lowest intracellular uptake and photodynamic activities which could point to an absence, or significantly lower densities of, receptors for lactose on the HT-29 cell line used in these experiments.

Conclusions

The two sets of phthalocyanines presented in this work, in which three different carbohydrate moieties (galactose, mannose and lactose) were grafted to functionalised monohydroxylated or monoazidophthalocyanines, respectively, by a direct glycosylation or a click linkage, were designed to investigate the effects of carbohydrate and linkage on photodynamic activity. The syntheses have been optimised, the carbohydrates being grafted on functionalised phthalocyanines of AB_3 type bearing three glycerol units in non-peripheral position which have a water-solubilising role. The systematic investigation of the nature of the carbohydrate showed, in accordance with previous works, that mannose-substituted phthalocyanines exhibit the best photodynamic activity. Nevertheless, cellular uptake experiments indicate that this is not due simply to a higher bulk internalisation of photosensitiser. The presence of the triazole linkage resulting from the click reaction had a negative effect on the mannose and galactose derivatives, but had no significant effect on the lactose conjugates, suggesting steric hindrance may be interfering with efficient recognition of the carbohydrate by receptors for the former two sugars, and a lack of significant receptor interactions for lactose on the HT-29 cell line.

Acknowledgement

The Scientific and Technological Research Council of Turkey TUBITAK (project Hızlı Destek 109T523) is gratefully acknowledged.

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